Thermal Acclimation Effects Differ between Voluntary, Maximum, and Critical Swimming Velocities in Two Cyprinid Fishes

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ABSTRACT

Temperature acclimation may be a critical component of the locomotor physiology and ecology of ectothermic animals, particularly those living in eurythermal environments. Several studies of fish report striking acclimation of biochemical and kinetic properties in isolated muscle. However, the relatively few studies of whole-animal performance report variable acclimation responses. We test the hypothesis that different types of whole-animal locomotion will respond differently to temperature acclimation, probably due to divergent physiological bases of locomotion. We studied two cyprinid fishes, tinfoil barbs (*Puntius schwanenfeldii*) and river barbels (*Barbus barbus*). Study fish were acclimated to either cold or warm temperatures for at least 6 wk and then assayed at four test temperatures for three types of swimming performance. We measured voluntary swimming velocity to estimate routine locomotor behavior, maximum fast start velocity to estimate anaerobic capacity, and critical swimming velocity to estimate primarily aerobic capacity. All three performance measures showed some acute thermal dependence, generally a positive correlation between swimming speed and test temperature. However, each performance measure responded quite differently to acclimation. Critical speeds acclimated strongly, maximum speeds not at all, and voluntary speeds uniquely in each species. Thus we conclude that long-term temperature exposure can have very different consequences for different types of locomotion, consistent with our hypothesis. The data also address previous hypotheses that predict that polyploid and eurythermal fish will have greater acclimation abilities than other fish, due to increased genetic flexibility and ecological selection, respectively. Our results conflict with these predictions. River barbels are eurythermal polyploids and tinfoil barbs stenothermal diploids, yet voluntary swimming acclimated strongly in tinfoil barbs and minimally in river barbels, and acclimation was otherwise comparable.

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Introduction

Temperature acutely affects the rates of most biological processes, from enzyme activities to whole-animal behavior. Rates typically accelerate with increasing temperature up to a maximum point or plateau and then rapidly decline (Cossins and Bowler 1987). The exact shapes of these response functions, or reaction norms, are frequently phenotypically plastic and modifiable with temperature acclimation (Cossins and Bowler 1987; Johnston and Bennett 1996). This combination of acute and acclimatory responses to temperature may critically influence the locomotor ecology of ectotherms, particularly those living in eurythermal environments. Much of our knowledge of this combination of acute and acclimatory effects focuses on biochemical and suborganismal physiological processes, particularly in fish (e.g., Kleckner and Sidell 1985; Sidell and Moerland 1989; Johnston et al. 1990; Hochachka and Somero 2002). Several fish studies also demonstrate that whole-animal locomotor capacities can respond to temperature acclimation (e.g., Griffiths and Alderdice 1972; Taylor et al. 1993; Johnson and Bennett 1995; Temple and Johnston 1998), but results are quite variable, and the broad patterns of this plasticity remain to be well explored.

Most research on whole-animal performance in fish examines either fast-start swimming, to obtain maximum speeds, or prolonged swimming, to obtain maximum sustainable speeds or endurance (Webb 1994; Reidy et al. 2000). Each measure theoretically estimates a maximum physiological capacity for performance, with fast-start swimming reflecting anaerobic capacity and prolonged swimming primarily aerobic capacity (Webb 1994; Reidy et al. 2000). These performance maxima should be important to fitness through effects on escaping predators and capturing prey, although these benefits are rarely measured (reviewed in Dommenici and Blake 1997). Far less studied than performance maxima are routine, motivationally regulated measures of swimming, such as spontaneous activity rates or voluntary speeds. These behaviors may impact fitness

through correlations with foraging efficiency or visibility to predators (McPeek 1990; Zhou and Weis 1999; Richardson 2001). Voluntary activity may also impact performance physiology, as activity rates may have training effects on maximal capacities (Davison 1997). A comprehensive understanding of temperature impacts on a species' locomotor physiology would benefit from documenting acute and acclimation responses of all three types of swimming performance (voluntary, fast start, and prolonged).

This comprehensive approach may be particularly important because different types of locomotion may respond differently to temperature. Voluntary, fast-start, and prolonged locomotion rely on overlapping but distinct physiological systems, and these differing physiological bases could contribute to differing responses to temperature. For instance, anaerobic energy use during activity is considerably less thermally dependent than is aerobic energy use (reviews in Bennett 1978, Bennett 1990), potentially resulting in reduced thermal dependence of anaerobic versus aerobically based locomotor performance. Previous studies of fast-start and prolonged swimming performance in fish report both the existence and the lack of acute and acclimation effects (reviewed in "Discussion"). These studies primarily measure only one type of locomotion each; thus, it remains unclear whether their diverse results are due to differences in protocols, temperature sensitivities of species, or temperature sensitivities of types of locomotion. The central goal of our study is to test the hypothesis that different types of locomotor performance, measured concurrently in the same study populations, will respond differently to temperature.

We secondarily address hypotheses that different species may have evolved different responses to temperature as a result of varying ploidy levels or ecology. The ploidy hypothesis suggests that polyploidy positively affects acclimation ability. Goldfish (*Carassius auratus*) and carp (*Cyprinus carpio*), both polyploid cyprinid fishes, show striking temperature acclimation of swimming performance and muscle biochemical and kinetic properties in contrast to several other fish that show minimal phenotypic plasticity of these traits (reviewed in "Discussion"; Johnson and Bennett 1995). Previous researchers suggest that the extra gene copies provided by polyploidy may allow the flexibility for different alleles to evolve adaptations to specific thermal environments (Sidell and Johnston 1985; Goldspink et al. 1992). The ecology hypothesis suggests that thermal ecology may also influence the evolution of swimming performance responses to temperature. Seasonal eurythermal habitats may create selection favoring reduced acute responses to temperature (to reduce effects of diurnal temperature fluctuation) but enhanced acclimation abilities (to reduce effects of seasonal temperature change; Kleckner and Sidell 1985; Temple and Johnston 1998). In contrast, stenothermal habitats may be selectively neutral or may favor stronger acute and reduced acclimation effects if physiological trade-offs restrict performance across temperatures (Huey and Hertz 1984). We examine the generality of both of these hypotheses by measuring acclimation patterns in two freshwater cyprinids that differ in ploidy, the diploid tinfoil barb *Puntius schwanenfeldii* and the tetraploid river barbel *Barbus barbus* (Taki et al. 1977; Arai 1982; Fister 1999). Tinfoil barbs are native to relatively stenothermal, tropical habitats in Southeast Asia (water temperature range 22°–25°C; Bailey and Cole 1999; McAdam et al. 1999), while barbels are native to eurythermal habitats across Europe (water temperature range 0°–25°C; Baras 1995). Thus, the ploidy and ecological hypotheses both predict that river barbels will show greater acclimatory abilities than tinfoil barbs.

In this study we measure acute and acclimatory temperature effects on voluntary swimming velocity, maximal velocity during fast starts, and critical swimming velocity, a measure of maximum sustainable speed, in both species of fish. We use these data to address the following four questions: (1) Do the three types of performance respond differently to acute temperature change? (2) Does temperature acclimation modify the acute response, and does acclimation response differ between performance types? (3) Do temperature responses differ between species? (4) Is acclimation beneficial?

Material and Methods

Study Subjects

We examined the influence of temperature in populations of two freshwater cyprinid fishes, tinfoil barbs *Puntius schwanenfeldii*, and river barbels *Barbus barbus*, both of the subfamily Cyprininae. We selected these species for their ecological and ploidy characteristics described above and because previous studies of the ploidy hypothesis have examined other cyprinids. We examined juvenile fish of both species (size range 4.5–6.5 cm standard length). The tinfoil barbs were obtained from a commercial tropical fish dealer who reported rearing the fish at a constant temperature of 23°C. The river barbels were obtained from a commercial fish farm in Great Britain. This supplier reported maintaining the barbels year round in outdoor tanks at environmental temperatures, which averaged 11°C at the time we obtained the fish. In our laboratory, all fish were maintained in 51 × 31 × 25 cm tanks at similar densities, were exposed to a 12L : 12D cycle, and were provided commercial fish food ad lib. once daily except on the day of an experiment, when fish to be tested were not fed. At each feeding, fish were observed to verify that they were eating, and all fish ate throughout the studies.

Temperature Acclimation

Tinfoil barbs were initially maintained at 23°C and river barbels at 11°C. After 1 wk we measured critical thermal limits of several fish of each species to identify appropriate acclimation temperatures. Critical thermal maximum and minimum were assayed as the loss of upright swimming ability during gradual

heating and cooling. A fish was placed in a 4-L beaker containing 3 L of water from their home tank and at the home temperature. Water in the beaker was heated or cooled at a rate of 0.3°C min^{-1}, and water temperature and fish behavior were monitored continuously. At the moment the fish lost upright orientation, temperature was recorded, and home temperature water was added. Loss of orientation was rapid and unequivocal, and recovery was immediate on addition of the home temperature water. Tinfoil barb critical maximum was 37.2° ± 0.3°C, and critical minimum was 12.6° ± 0.2°C (mean ± SE, $n = 5$ each). River barbel critical maximum was 29.2° ± 0.2°C, and critical minimum was 0.4° ± 0.1°C ($n = 3$ each).

We selected acclimation temperatures of 4°C above and below the measured critical thermal minima and maxima, respectively, except for the river barbel cold-acclimation treatment. River barbels did not feed at 4°C and so were cold acclimated to 7°C, where they fed readily. Barbels were warm acclimated to 25°C and tinfoil barbs acclimated to either 17° or 33°C. To achieve these temperatures, three tanks per species per treatment were gradually cooled or heated over a 2-wk period to the target acclimation temperatures. Fish were then maintained at acclimation temperature for a minimum of 6 wk before swimming performance tests.

Acute Temperature Tests

Swimming performance of tinfoil barbs was tested at 17°, 20°, and 30°C in 17°C-acclimated fish and at 20°, 30°, and 33°C in 33°C-acclimated fish. Tinfoil barbs were not measured at the opposite acclimation temperatures as neither acclimation group would swim at the opposite temperature. The test temperatures of 20° and 30°C were selected as those nearest acclimation temperatures at which both groups swam readily. In river barbels, both acclimation groups swam at the opposite temperature, and performance in this species was measured at 7°, 13°, 19°, and 25°C. Temperatures in all testing environments were continuously monitored and varied less than 0.5°C. For each experiment we used eight to 11 fish per acclimation group (two to four fish from each acclimation tank), and size matched fish within and among groups.

Our protocols were designed to provide conservative estimates of acute temperature effects on swimming performance. Acute temperature change may inevitably create stress that may impact performance, and stress might be greater the greater the temperature change. We endeavored to reduce stress by allowing significant adjustment periods to test temperatures (protocols below). However, we also measured individual fish at multiple test temperatures in randomized block designs, dividing each acclimation group into equal subgroups that encountered the different test temperatures in different order. In these designs, any influence of the degree of temperature change or of testing order on swimming performance would increase the variance in the data but not create bias. Due to the potentially increased variance, subsequent statistical comparisons of performance at different temperatures would produce conservative conclusions.

To help control variance in our comparisons between different types of swimming performance, we measured two of the three performance types in concert. Voluntary and fast-start swimming speeds were measured in an individual fish in a single testing arena on the same day at each test temperature. These tests exposed fish to a maximum of eight fast-start tests (duration >0.5 s each) over a minimum of 375 min, a protocol that in our experience results in no reduction in fast-start or voluntary swimming speeds as a function of time. In contrast, measures of critical speed require fish to swim at increasing speeds for up to several hours until exhaustion. To reduce the potential influence of fatigue, critical speed tests were conducted separately from the voluntary and fast-start tests, and fish were given 3 d rest between critical speed tests at different temperatures.

Voluntary Swimming Speed

We recorded voluntary swimming speeds of individual fish using a Chromotrack video-tracking system (San Diego Instruments, San Diego, Calif.). The system tracked horizontal movement of fish as they swam within a 32 × 25 cm enclosure in water 7 cm deep. Vertical movement was minimized by the relatively shallow water, and the white plastic enclosure blocked external visual disturbance and optimized tracking conditions. A video camera connected to a computer was positioned directly above the tank. Every 0.055 s, Chromotrack software recorded Cartesian coordinates (±1.4 mm) of the upper-leftmost point of the fish, as seen from above, and calculated the distance traveled by this point over time. From these data we estimated mean swimming speeds of fish during 30-min recordings.

Voluntary swimming of individual tinfoil barbs was measured at each of two temperatures, 20° and 30°C, during a single day. At the start of a test, the temperature of the testing tank was set to 20°C for 17°C-acclimated fish and 30°C for 33°C-acclimated fish. A fish was placed in the testing tank in a plastic bag containing 500 mL of water from the fish's acclimation tank, was left in the bag 15 min to allow temperature to equalize, and was then released. In half of the trials, water temperature was kept stable while the fish was left undisturbed for 40 min and then video recorded for 30 min. In the other half of the trials, water temperature was gradually changed to the other test temperature over 40 min following fish release. The fish was then left undisturbed for 40 min and then video recorded for 30 min. At the end of the first voluntary swimming recording, fast-start performance was measured. Water temperature was then changed to the second test temperature over 40 min, the fish were left undisturbed for another 40 min and

then video recorded for 30 min, followed by a second fast-start measurement. Finally, the test tank was returned to the acclimation temperature of the subject, and the subject was returned to its acclimation tank. A separate set of tinfoil barbs was measured at their acclimation temperatures only, to provide a formal test of the beneficial acclimation hypothesis. Data from these trials were not used in the overall analyses of acclimation and acute temperature effects. In these trials the test tank was set to the subject's acclimation temperature, the subject was left undisturbed for 40 min and then video recorded for 30 min, and then fast-start measured.

Voluntary swimming of river barbels was measured in a similar fashion except that each barbel was measured at each of four temperatures per trial. Eight fish from each acclimation group were assayed, with two fish from each group being tested in one of four testing orders: (1) 25°, 19°, 13°, 7°C; (2) 19°, 25°, 7°, 13°C; (3) 13°, 7°, 25°, 19°C; (4) 7°, 13°, 19°, 25°C. Thus, for each acclimation group, each test temperature was used an equal number of times at each position in the testing order. We regard these measurements of voluntary swimming as voluntary exploratory behavior in a simple novel environment: they are not meant to simulate voluntary speeds in nature.

Maximum Fast-Start Speed

We assayed maximum swimming speeds using high-speed video to record fast- or "C-start" responses to a standardized stimulus. The fast start is a stereotyped escape response found in nearly all teleost fishes, in which the fish first bends its body into a C-shape about the center of its mass by contracting all the muscle fibers on one side of its body and then accelerates with a propulsive stroke of its tail (reviews in Webb 1994; Dommenici and Blake 1997; Hale 2000). We recorded fast starts following the voluntary swimming recordings using a high-speed video camera (NAC model HSV-500 recording at 250 frames s^{-1}) placed directly above the tank. We elicited a fast start by releasing a small weight that was suspended inside a vertical, opaque plastic tube with its bottom opening under water within the fish enclosure. Two fast starts were recorded for each fish at each temperature with 15 min rest between each start.

For each fast start we analyzed 25 consecutive frames of videotape (100 ms total) starting with the frame preceding the first detectable movement of the C-start. The 25 frames were saved to an IBM PC using a Videoblaster frame grabber (Creative Labs, Milpitas, Calif.). We analyzed movement using custom software to digitize a set of 10 points tracing the midline of the fish (from top view) in each video frame. The software recorded Cartesian coordinates for each digitized point. We transferred the resulting tables of coordinates to Microsoft Excel and used a customized macro to determine rate of displacement of the center of mass of the fish, the focal point for successful predator attacks in several prey fishes (Webb 1986). The center of mass was identified as the point along the fish's midline that was the center of bending during the initial phase of the fast start. Maximum fast-start speed was defined as the maximum speed of the center of mass during any 8-ms interval of the 100-ms recording.

Critical Swimming Speed

Endurance or prolonged swimming performance was measured as critical swimming speed (Brett 1964; Beamish 1978; Hammer 1995). In this test a fish is placed in a flow tank and allowed to swim for a set time interval at a given speed. Speed is then increased by a standard increment, held constant for the set time interval, and the process is then repeated until the fish refuses to continue swimming. Critical swimming speed is calculated as $V_p + [(t_f/t_{in}) \times V_{in}]$, where V_p is the penultimate speed at which the fish swam before exhaustion, t_f is the time between the final speed increase and exhaustion, t_{in} is the time interval between speed increases (here 15 min), and V_{in} is the increment of speed increase (here 0.05 m s^{-1}; Brett 1964; Beamish 1978; Hammer 1995).

We measured critical speed in a flow tank modeled after Vogel and LaBarbera (1978). The section of the flow tank used for the experiments measured 120 cm long and 14 cm wide and contained water 13 cm deep. A swimming cage 35 cm long, 10 cm wide, and 14 cm high was suspended 3 cm off the bottom of the tank in the middle of this section to restrain fish within the main flow. The cage's upstream end and sides consisted of thin mesh and the downstream end of a plastic "egg crate" collimator (plastic grid 1 cm deep with grid squares 1 cm square). Two collimators streamlined the water flow upstream of the cage (Vogel and LaBarbera 1978). Downstream of the cage, a large airstone kept the water aerated. Flow rate was controlled by a variable speed motor (Bodine Electric, Chicago) driving a stainless steel propeller located downstream of the swimming cage. Motor speed was regulated using a voltage controller (Minarik Electric, Los Angeles). We calibrated water flow rate using the high-speed video (250 frames s^{-1}) to record movement of a dye suspension through the flow tank over the total range of speeds used. Dye was injected upstream of the swimming area through a needle bent at 90° so that the dye was injected parallel to the water flow. Water flow was linearly correlated with voltage control of the propeller (linear regression of voltage controller setting and measured water flow rate; $n = 15$, $R^2 = 0.994$, $P < 0.0001$).

On the day of a trial, a fish was placed in the flow tank in a bag with 1 L of water from the acclimation tank and an active airstone for a minimum of 2.5 h to allow temperature equalization and initial adjustment. The fish was then released into the swimming cage with the water flow rate at 0. After 15 min, flow was initiated at a very low rate to allow the fish to orient to the flow. For tinfoil barbs this initial rate was 0.140 m s^{-1}, and this rate was maintained for 15 min. Flow was then grad-

ually increased over 1 min to 0.282 m s^{-1}, and the trial was begun, with flow increasing an additional 0.052 m s^{-1} every 15 min. River barbels took longer to settle into steady swimming in the flow tank and required a slightly longer orientation protocol. After 15 min in the tank at zero flow, the flow for river barbels was initiated at 0.101 m s^{-1}, was maintained there for 10 min, and then was increased to 0.179 m s^{-1} and maintained there for an additional 15 min. Flow was then gradually increased over 3 min to 0.334 m s^{-1} (the second speed step in the tinfoil trials) and the trial was begun, with flow increasing 0.052 m s^{-1} every 15 min, as above.

Once the trial starting speed was reached, fish of both species typically swam steadily against the flow. Fish were discouraged from butting or bracing themselves against the rear of the cage by a transient electric field. A parallel series of fine silver wires was secured to the rear plastic grid and connected to a voltage regulator, allowing generation of the field. The field was turned on only when a fish appeared to be bracing its tail against the rear grid and was kept on for only 2 s. The field was kept off for 60 s any time a fish made contact with the field three times within 60 s or appeared at risk of touching the field with any part of the body besides the tail. Fish typically avoided the rear grid until near exhaustion. The trial was concluded when a fish remained trapped against the grid by the flow of water for 10 s. All fish resumed normal swimming behavior as soon as flow was stopped.

We measured the critical swimming speed of 17°C-acclimated tinfoil barbs at 17°, 20°, and 30°C, and 33°C-acclimated tinfoil barbs at 20°, 30°, and 33°C. River barbels from both acclimation groups were measured at 7°, 13°, 19°, and 25°C. Each individual was measured at all test temperatures designated for its group and was given a minimum of 3 d rest between tests in their home acclimation tanks. The order of test temperatures was diversified within and controlled across acclimation groups in both species by using the method described above for river barbel voluntary swimming. Several additional fish of both species were measured at a single test temperature each, and several tinfoil barbs were measured at 25°C. Critical speeds for these one-time measured fish fell within one standard deviation of the mean data for repeated-measures fish at the same temperatures. Data for all fish are presented in the figures. We used repeated-measures statistics and present analyses for fish measured at repeated temperatures only. Non-repeated-measures analyses using all data gave the same results and for simplicity are not shown.

Statistical Analyses

The three swimming experiments examined locomotor performance as a function of both acclimation and test temperatures. The data were analyzed using two-way repeated-measures (RM) ANOVA that included the main effect acclimation group (error term = subjects within groups), the repeated effect test temperature (error term = test temperature × subjects within groups), and the interaction of acclimation group × test temperature (error term = test temperature × subjects within groups; SAS 1999). In the tinfoil barbs, swimming performance of both acclimation groups was measured at two common test temperatures, 20° and 30°C, and therefore the main RM ANOVAs for tinfoil barbs included only these two test temperatures. In the river barbels, swimming performance of both groups was tested using four common test temperatures, 7°, 13°, 19°, and 25°C, and all models included all four test temperatures. When the main models detected interaction effects, additional RM ANOVAs were constructed within acclimation groups as post hoc tests for identifying the nature of the interaction. These models included all repeated test temperatures used for that acclimation group, which in tinfoil barbs was two per group for voluntary and fast-start swimming and three per group for critical swimming speed, and for river barbels was always four temperatures per group.

We used one-way ANOVA to compare swimming performance between acclimation groups when each was swimming at their own acclimation temperature (to test for complete compensation). All of our analyses were initially constructed including fish standard length as a covariate. Length was not significant in any of the models, probably due in part to close size matching within experiments, and we excluded length from the final models. Statistical analyses were generated on a Macintosh computer using Statview and SuperANOVA software (SAS Institute, Cary, N.C., and Abacus Concepts, Berkeley, Calif.).

Results

Voluntary Swimming

Temperature had very different effects on voluntary swimming speed in the two species of fish. In tinfoil barbs, warm-acclimated fish swam at a speed over twice that of cold-acclimated fish when tested at 20°C (Fig. 1*A*), resulting in a significant main effect of acclimation temperature (Table 1A). However, both acclimation groups swam at intermediate speeds when tested at 30°C (Fig. 1*A*), resulting in an acclimation × test temperature interaction effect (Table 1A). Thus, the two acclimation groups responded differently to test temperature, an effect further illustrated by post hoc analyses. In the main analysis, test temperature showed no effect on voluntary swimming speed (Table 1A). Yet post hoc analyses constructed within each acclimation group found test temperature effects in each group, indicating that speed of cold-acclimated tinfoil barbs significantly increased between 20° and 30°C, while speed of the warm-acclimated group decreased between the same temperatures (Fig. 1*A*; Table 2; RM ANOVAs, cold group, $F_{1,10} = 8.2$, $P = 0.017$; warm group, $F_{1,10} = 4.8$, $P = 0.05$). Finally, warm-acclimated tinfoil barbs had faster voluntary swimming speeds at their acclimation temperature than did cold-accli-

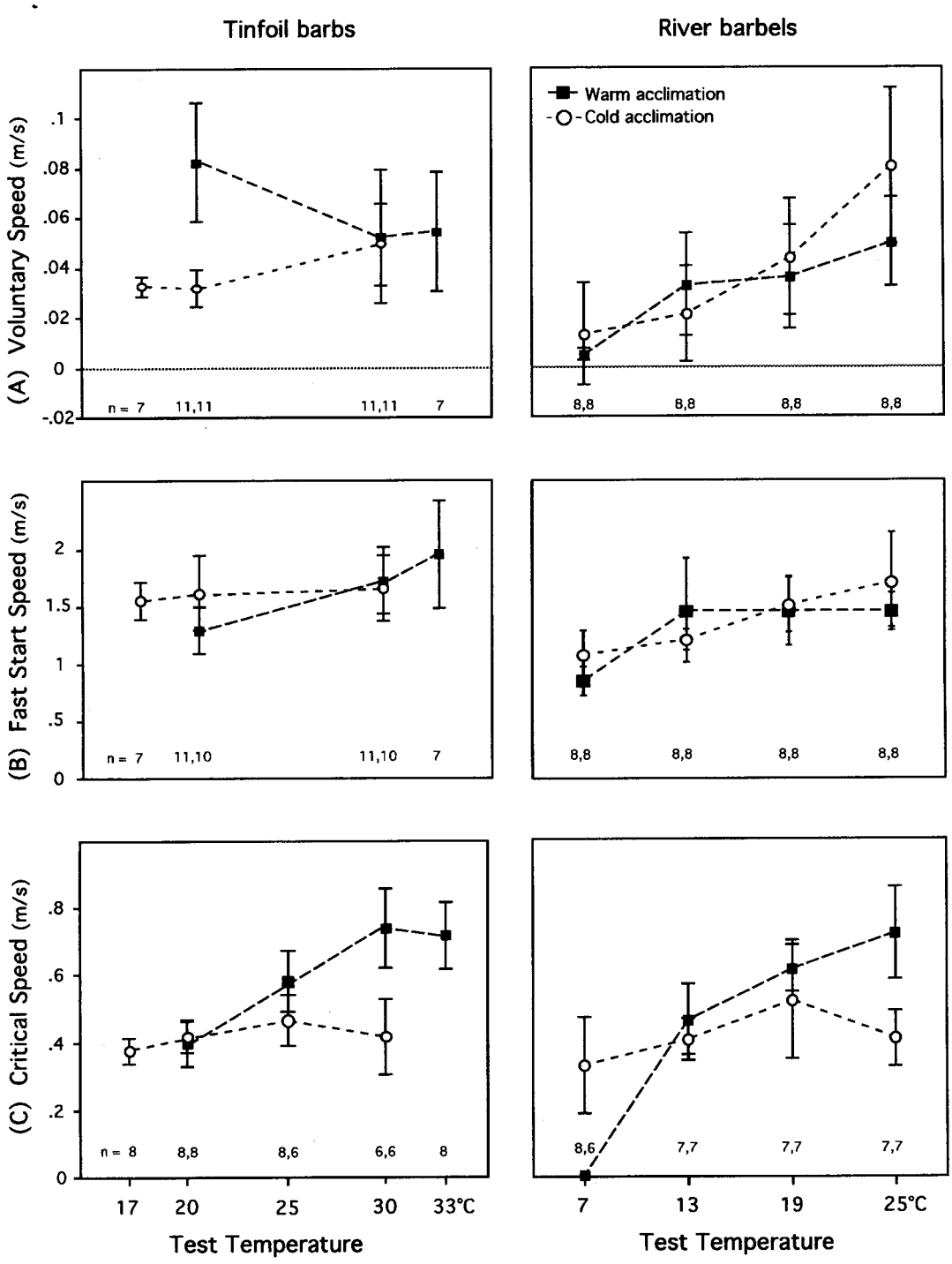

Figure 1. Effects of temperature acclimation and acute (test) temperature on swimming performance of tinfoil barbs *(Puntius schwanenfeldii)* and river barbels *(Barbus barbus)*. *A*, Acclimation altered the acute response of voluntary speed, a measure of routine swimming behavior, to test temperatures. In tinfoil barbs, voluntary speed was negatively correlated with test temperature in warm-acclimated fish but positively correlated in cold-acclimated fish. In contrast, in river barbels voluntary speed was positively correlated with test temperature in both acclimation groups, but the correlation was steeper in cold-acclimated fish. *B*, Maximum fast-start speed was unaffected by acclimation in both species. Maximum speed also did not vary with test temperature in tinfoil barbs but was positively correlated with test temperature in river barbels. *C*, Acclimation of critical swimming speed, a measure of endurance, altered acute responses to temperature and did so similarly in both species. Test temperature was positively correlated overall with critical speed, but the response curve was shifted to the right and was higher peaked in warm- compared with cold-acclimated fish. Table 1 presents statistics. n = sample size. At test temperatures where two groups were measured, n for the warm-acclimated group is listed first. Error bars indicate 95% confidence intervals.

Table 1: Repeated-measures ANOVAs of swimming performance

		Tinfoil Barbs			River Barbels		
Dependent	Source of Variation	df	*F*	*P*	df	*F*	*P*
A. Voluntary swimming speed	Acclimation temperature	1	6.7	**.018**	1	.9	.35
	Error: subject (group)	20			14		
	Test temperature	1	.6	.44	3	21.3	**.0001**
	Test temperature × acclimation temperature	1	10.2	**.005**	3	2.9	**.049**
	Error: test temperature × subject (group)	20			42		
B. Maximum fast-start speed	Acclimation temperature	1	2.4	.14	1	.9	.35
	Error: subject (group)	19			14		
	Test temperature	1	2.4	.13	3	9.8	**.0001**
	Test temperature × acclimation temperature	1	1.6	.22	3	1.8	.17
	Error: test temperature × subject (group)	19			42		
C. Critical swimming speed	Acclimation temperature	1	14.2	**.004**	1	.9	.37
	Error: subject (group)	10			7		
	Test temperature	1	29.8	**.0003**	3	81.8	**.0001**
	Test temperature × acclimation temperature	1	31.2	**.0002**	3	40.6	**.0001**
	Error: test temperature × subject (group)	10			21		

mated fish at their acclimation temperature (one-way ANOVA, $F_{1,12} = 5.0$, $P = 0.046$).

In contrast, voluntary swimming in river barbels showed no overall response to acclimation but did vary with test temperature, as warm- and cold-acclimated river barbels both increased their voluntary speeds as test temperature increased (Fig. 1*A*; Table 1A). In addition, the shape of this positive relationship differed slightly between groups, apparently due to cold fish showing lower thermal dependence (Q_{10}) at colder test temperatures and warm fish showing lower Q_{10} at warmer temperatures (Fig. 1*A*; Table 1A, test × acclimation temperature interaction). When measured at their own acclimation temperatures, warm-acclimated barbels swam nearly four times faster than cold-acclimated fish (Fig. 1*A*; one-way ANOVA: $F_{1,15} = 9.4$, $P = 0.008$).

Maximum Fast-Start Speed

Acclimation had no effects on fast-start swimming in either species of fish (Fig. 1*B*; Table 1B). Both species also showed little variation in speed over the range of test temperatures, with river barbels showing a slight positive correlation, and neither species showing a difference between acclimation groups in response to test temperature (Fig. 1*B*; Table 1B). However, comparisons of maximum speed of fish measured at their acclimation temperatures showed that performance was marginally greater in warm- than in cold-acclimated tinfoil barbs ($F_{1,12} = 4.4$, $P = 0.058$) and significantly greater in warm- than cold-acclimated river barbels ($F_{1,14} = 11.8$, $P = 0.004$).

Critical Swimming Speed

Temperature showed pervasive and similar effects on our endurance measure in both fish species. In tinfoil barbs, critical speed of warm-acclimated fish was strongly positively correlated with test temperature, resulting in an overall influence of test temperature in this species (Fig. 1*C*; Table 1C). However, critical speed of cold-acclimated tinfoil barbs showed an almost flat relationship with test temperature, resulting in lower overall speeds than the warm-acclimated fish and thus significant acclimation effects and interactive effects of acclimation and test temperatures (Fig. 1*C*; Tables 1C, 2). Post hoc analyses within acclimation groups elucidated the interaction term, indicating that cold-acclimated tinfoil barbs indeed showed no detectable variation in critical speeds across test temperatures (RM ANOVA, $F_{2,10} = 1.2$, $P = 0.34$), while warm-acclimated fish showed steeply increasing speeds with increasing temperature ($F_{2,10} = 40.2$, $P = 0.0001$). Predictably, therefore, when measured at their own acclimation temperatures, warm-acclimated tinfoil barbs had much higher critical speeds than did cold fish (one-way ANOVA, $F_{1,14} = 58.4$, $P = 0.0001$; Fig. 1*C*).

Critical swimming speeds of river barbels displayed similar patterns. Warm-acclimated barbels showed a steeply positive effect of test temperature on critical speed, while cold-acclimated fish showed a flatter relationship and lower mean speeds, except at 7°C (Fig. 1*C*). This pattern is reflected in significant main effects of test temperature and interactive effects of test × acclimation temperature on barbel critical speed (Table 1C). Acclimation temperature showed no main effect, but this result is due to the zero-speed performance of the warm-acclimated fish at 7°C (Fig. 1*C*). Exclusion of the 7°C measurements from the analysis resulted in significant acclimation main

Table 2: Temperature coefficients (Q_{10}) for swimming performance

	Tinfoil Barbs		River Barbels	
	Cold (17°C) Acclimation	Warm (33°C) Acclimation	Cold (7°C) Acclimation	Warm (25°C) Acclimation
Voluntary speed	1.67	.67	5.98	2.57
Fast-start speed	1.17	1.43	1.43	1.42
Critical speed	1.01	1.86	1.18	1.68

Note. Tinfoil barb Q_{10} calculated from 20° to 30°C. River barbel fast-start Q_{10} calculated from 7° to 25°C. River barbel voluntary and critical speed Q_{10} calculated from 13° to 25°C due to zero-value measures at 7°C. All calculations from mean values for data presented in Figure 1.

effects (analysis not shown). Post hoc tests indicated that test temperature affected critical speed within both acclimation groups of river barbels (RM ANOVAs: cold fish, $F_{3,9} = 10.1$, $P = 0.0031$; warm fish, $F_{3,12} = 102.6$, $P = 0.0001$) and thus that the interaction effect in the main analysis was due to a higher Q_{10} in warm- versus cold-acclimated fish (Table 2). When measured at acclimation temperatures, warm-acclimated river barbels showed over double the critical speed of cold (one-way ANOVA, $F_{1,11} = 50.7$, $P = 0.0001$).

Discussion

Consistent with our hypothesis, each type of swimming performance displayed a unique response to temperature acclimation, with critical speed acclimating the most and maximum speed not at all. In contrast, all performance types showed some acute thermal dependence, usually a positive correlation between swimming speed and test temperature. Yet the slope of this correlation often differed between acclimation groups. The pattern of these differences in slopes was unique for each type of swimming performance (Fig. 1), also consistent with our hypothesis. We discuss these patterns below, addressing possible genetic, physiological, and ecological explanations for the variation in performance responses to temperature.

Acute Thermal Dependence of Swimming Performance

Acute thermal dependencies of fast-start and critical swimming speeds may result in part from thermal effects on anaerobic and aerobic metabolic capacities, respectively (Bennett 1990). However voluntary swimming differs from more common measures of performance in that it should be relatively unaffected by such constraints. Voluntary speeds were 1/10 of critical speeds and 1/20 of fast-start speeds in both species in our study (Fig. 1) and thus well below possible physiological or biomechanical limits on performance. Motivational factors may instead primarily influence voluntary swimming. For example, selection may favor increased spontaneous activity in response to acute temperature change in fish that live in mainly stenothermal or locally eurythermal habitats and may be able to locate preferred thermal habitat predictably (Stevens 1973). Previous studies do document increased activity rates following acute temperature change, and active behavioral thermoregulation, in several fish (reviews in Stevens 1973; Baras 1995). These results correspond to the increased activity that we observed in tinfoil barbs confronting novel thermal environments (Fig. 1*A*), and tinfoil barbs are native to relatively stenothermal habitats (Bailey and Cole 1999; McAdam et al. 1999). In contrast, fish living in environments with circadian or seasonal temperature cycles may not be able to locate preferred temperatures. These habitats might favor quite varied relationships between voluntary activity and acute temperature, depending on relationships among performance capacity, predation risk, resource availability, and temperature. River barbels are native to eurythermal habitats in which they greatly reduce activity during winter months and to a lesser extent during times of day when temperatures are below preferred levels (Baras 1995). This positive correlation between field activity and temperature is similar to our laboratory result (Fig. 1*A*) and to laboratory studies of spontaneous activity in three other eurythermal fish (Crawshaw 1984; Fuiman and Ottey 1993). These positive relationships might function to reduce activity when maximal performance abilities are compromised (Fig. 1*C*; Beamish 1978). Notably, however, these hypothesized ecological associations are almost entirely unresearched. Thermal dependencies of voluntary swimming vary considerably among the limited existing studies, and any ecological, phylogenetic, or physiological patterns in this variation await documentation.

Unlike voluntary swimming, the acute thermal dependence of maximum swimming speed appears relatively predictable and influenced by physiological constraints. Fast-start speed responded to temperature similarly in tinfoil barbs and river barbels (Fig. 1*B*), and our results fall within the spectrum of the flat to strongly positive thermal dependence previously reported for other species of fish (e.g., Beddow et al. 1995; Johnson and Bennett 1995; Temple and Johnston 1998). Body temperature may constrain maximum speed via effects on the biomechanical and contractile properties of fast-twitch muscle (Bennett 1990; Hochachka and Somero 2002). This possible constraint is supported by correlational evidence. In three species of fish, myofibrillar ATPase activities and twitch contraction times of white epaxial muscles showed thermal dependencies similar to that of C-start kinetics, which ranged from flat to

strongly positive (Johnson and Bennett 1995; Johnson et al. 1996).

The acute response of critical swimming speeds also appears moderately predictable. Our finding of a generally positive correlation with test temperature up to a maximum (Fig. 1*C*) is similar to response curves reported for most other fish (reviews in Beamish 1978, 1981; Hammer 1995), though not all (Schneider and Connors 1982). Critical speed is generally believed to be subject to aerobic physiological constraints, consistent with its being used as a measure of sustainable performance capacity. However, identifying the physiological factors regulating critical speed has proved quite elusive (Kolok and Farrell 1994; Webb 1994; Hammer 1995; Reidy et al. 2000). Critical speeds were positively correlated with metabolic scope in cod (*Gadus morhua*; Reidy et al. 2000) and can be increased by training in many fish (reviewed in Davison 1997) but were uncorrelated with condition, cardiac physiology, and metabolic parameters in several other fishes (Kolok and Farrell 1994; Hammer 1995; Nelson et al. 1996; Reidy et al. 2000). Thus, while the shape of the acute thermal response of critical speed seems predictable, the physiological factors that underlie the relationship remain obscure.

Acclimation of Swimming Performance

Within each of our two study species, the three types of swimming performance responded very differently to temperature acclimation. We were particularly curious about the response of voluntary swimming, as almost no previous studies have examined acclimation of this behavior. We found a strong acclimation response in tinfoil barbs (Fig. 1*A*) and hypothesize that acclimation may have changed the preferred body temperatures of these fish, influencing their activity levels in part as a consequence of thermoregulatory behavior. However, changed temperature preferences cannot broadly explain acclimation of voluntary swimming, as river barbels showed a small and opposite acclimation pattern. Interestingly, a study of spontaneous activity in red drum *(Sciaenops ocellatus)* reported yet a third acclimation pattern that also suggests no influence of thermoregulatory behavior. Red drum activity was positively correlated with test temperature in both 21°- and 26°C-acclimated fish, but the 26°C acclimation group showed higher activity at every test temperature (Fuiman and Ottey 1993). These three sets of results demonstrate that motivationally based measures of performance can indeed acclimate but that the form of the response is very unpredictable.

Acclimation of voluntary activity should merit further study in a physiological context because voluntary locomotion may directly influence exercise physiology and so contribute to acclimation patterns of other levels of performance. Training improves swimming stamina in many teleosts (Davison 1997), and indeed activity rates are positively correlated with activity capacity in the majority of vertebrates studied (e.g., Cummings 1979; Miller and Camilliere 1981; Mazzeo et al. 1998; Swallow et al. 1998). In our study temperature acclimation apparently influenced daily voluntary swimming rates. When measured at their own acclimation temperatures, cold-acclimated fish of both species displayed much slower voluntary speeds than warm-acclimated fish (e.g., cold-acclimated barbels swam at 0.01 ± 0.01 m s^{-1} at 7°C, while warm-acclimated barbels swam at 0.05 ± 0.01 m s^{-1} at 25°C, mean ± SE; Fig. 1*A*). These results correlated with our daily anecdotal observations that warm-acclimated fish were much more active than cold-acclimated fish in their home tanks. Higher activity rates in warm-acclimated fish may have had a training influence that contributed to their greater maximum critical speeds (Fig. 1*C*; see "Results"). A previous study supports this possibility. After thermal acclimation, warm-acclimated trout (*Oncorhynchus mykiss*) displayed higher critical speeds than cold-acclimated fish when each group was measured at their own acclimation temperature, a result similar to ours (Kieffer et al. 1998). However, this difference disappeared after both acclimation groups completed 2 wk of training (swimming at 0.1 m s^{-1}; Kieffer et al. 1998).

Thermal acclimation of maximum swimming speed was interesting for its lack of effects (Fig. 1*B*; Table 1). Previous studies of acclimation effects on fast-start kinetics document a wide spectrum of responses, ranging from goldfish (*Carassius auratus*), which have large shifts in C-start kinetics following temperature acclimation (Johnson and Bennett 1995), to killifish (*Fundulus heteroclitus*), which display smaller but still notable shifts (Johnson and Bennett 1995), to several other species that have little discernible acclimation effect (Johnson et al. 1996; Temple and Johnston 1998; Temple et al. 2000; Wilson et al. 2001). Our results fall into the latter part of this spectrum. Additional studies suggest that fast-start responses to acclimation are potentially limited by biochemical and physiological properties of white (fast-twitch) muscle (reviews in Sidell and Moerland 1989; Guderley et al. 2001). The larger acclimation response of goldfish is reflected in changes in myosin heavy chain isoform expression, myofibrillar ATPase activity, and white muscle twitch contraction kinetics not present in killifish or trout (*O. mykiss*; Johnson and Bennett 1995; Johnson et al. 1996). A similar study of tinfoil barbs and river barbels found acute thermal effects but no acclimation effects on white muscle myofibrillar ATPase activity and no change in myosin isoform expression with acclimation, supporting the correspondence between organismal and suborganismal acclimation of fast starts (O'Steen et al. 1999).

In contrast to the other performance measures, critical swimming speed displayed strong acclimation responses in both of our study species. Relatively few previous studies have examined acclimation effects on critical speed. Research on carp (*Cyprinus carpio*) found responses quite similar to those of tinfoil barbs and river barbels; cold-acclimated carp showed much lower Q_{10}'s than warm-acclimated fish, and each acclimation group showed the greater critical speed at its own ac-

climation temperature (Heap and Goldspink 1986). A study of critical speed in trout (*O. mykiss*) found potentially similar results in that critical speed measured at acclimation temperatures was higher in warm- than cold-acclimated fish (Kieffer et al. 1998). However, speeds were measured only at acclimation temperature, and so acute and acclimation effects could not be differentiated. A study of smelt (*Hypomesus transpacificus*) also measured critical speeds only at acclimation temperatures but reported a distinctly different pattern, as critical speeds were the same at 12°, 17°, and 21°C (Swanson et al. 1998). Thus, smelt either display perfect compensation in response to acclimation or lack any acute temperature response—both unique results. Critical speed clearly acclimates strongly and similarly in several species, but some species differences may exist, and thus phylogenetic studies of acclimation patterns may reward further study. The small number of critical speed acclimation studies limits understanding of its physiological basis, though endurance acclimation must be partially influenced by suborganismal acclimation of aerobic functions (Sidell and Moerland 1989). Consistent with this inference, carp show striking acclimation of contractile properties, protein isoform expression, and aerobic enzyme activities of both white and red muscle (Johnston et al. 1985; Crockford and Johnston 1990; Fleming et al. 1990).

Patterns across Species: Polyploidy and Acclimation

Previous work demonstrates that goldfish have unusually large responses to temperature acclimation that are consistent across molecular, biochemical, cellular, and organismal levels (Johnson and Bennett 1995). Carp show similarly large responses of suborganismal processes (Sidell and Moerland 1989; Crockford and Johnston 1990; Fleming et al. 1990; Johnston et al. 1990) and of critical speed (Heap and Goldspink 1986). Carp and goldfish are both polyploid cyprinids, and the ploidy hypothesis suggests that their striking acclimation abilities result from their possession of multiple gene copies (reviewed in Johnson and Bennett 1995). The hypothesis predicts that polyploidy and exceptional acclimatory ability should co-occur, but our results do not support this prediction. Tinfoil barbs are diploid and river barbels polyploid cyprinids (Taki et al. 1977; Arai 1982; Fister 1999), yet both displayed similar and unexceptional acclimation of fast-start and critical swimming. Tinfoil barbs and barbels differed in the acclimation of voluntary swimming, but here the diploid tinfoil barb displayed the larger response. Additionally, previous work on trout indicates that this polyploid salmonid also lacks exceptional acclimatory abilities across several levels of biological organization (Johnson et al. 1996). While insufficient studies exist to identify broad patterns, the available data are notably inconsistent with the ploidy hypothesis, even among cyprinids.

Adaptive Benefits of Acclimation

In the last decade, hypotheses predicting the adaptive benefits of temperature acclimation have evolved rapidly and have displayed several illuminating branching events. These hypotheses include beneficial acclimation, which formalizes a long-standing physiological assumption that acclimation should improve fitness at the acclimation temperature (Leroi et al. 1994); optimal temperature, which suggests there is an optimal developmental or acclimation temperature that maximizes fitness (Zamudio et al. 1995); and hotter is better, which suggests that fitness should generally improve with increasing developmental or acclimation temperature (Huey et al. 1999; van Damme and Vanhooydonck 2001) and is a basis for one theory on the evolution of endothermy (Crompton et al. 1978; Stevens and Carey 1981; Block et al. 1993). One difficulty in testing these hypotheses is quantifying the fitness consequences of acclimation. Acclimation is typically studied in the laboratory, where even studies that directly measure reproductive fitness must assume their results are relevant in nature, and most studies measure performance, such as enzyme activities or swimming speed, as proxies for fitness. A second difficulty is that differentiating between the hypotheses requires at least a three-acclimation by three-test temperature experimental design (3×3), which is rarely used (Huey and Berrigan 1996). Huey and colleagues (Huey and Berrigan 1996; Huey et al. 1999) review these challenges and a series of studies that fulfill at least the second criterion. They conclude that no one hypothesis is uniquely supported, that instead, each has some importance, and the optimal temperature hypothesis enjoys the greatest support (Huey et al. 1999).

Our results, albeit constrained by the above limitations, provide similarly mixed support for these hypotheses. We assume that higher fast-start and critical swimming speeds represent benefits to fitness (support for fast starts in Watkins 1996), but we exclude discussion of voluntary swimming because we have no grounds for assuming high versus low speeds improve fitness. We used a 2×4 experimental design, and therefore we should be able to distinguish beneficial acclimation from the optimal temperature and hotter-is-better hypotheses but not differentiate the latter two. Our data on tinfoil barbs generally support beneficial acclimation in that both acclimation groups refused or were unable to swim at the other groups acclimation temperature (Fig. 1; see "Material and Methods"). Thus acclimation apparently benefited performance of tinfoil barbs at acclimation temperature but at the cost of reduced tolerance to the opposite thermal extreme. River barbel critical speed data also support beneficial acclimation, as both acclimation groups outperformed the other at home acclimation temperatures (Fig. 1*C*; see "Results"). However, barbel fast-start speed data do not support beneficial acclimation, as acclimation groups showed similar performance at all temperatures (Fig. 1*B*; Table 1B), nor do they support the hotter-is-better and

optimal temperature hypotheses, for the same reason. Our only support for the hotter-is-better or optimal temperature hypotheses derives from the critical speed results. These results primarily support beneficial acclimation. However, they are not entirely inconsistent with hotter is better or optimal temperature, as in both species, warm-acclimated fish generally out performed cold-acclimated fish (Fig. 1*C*; Table 1C; see "Results"). We conclude that while our findings are most consistent with the beneficial acclimation hypothesis, they more generally support Huey et al.'s (1999; Huey and Berrigan 1996) conclusion that elements of each hypothesis may contribute to the adaptive benefits of temperature acclimation when benefits occur. Similarly, our findings support Huey and Berrigan's (1996) suggestion that any broad conclusions await field studies of the benefits of acclimation.

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